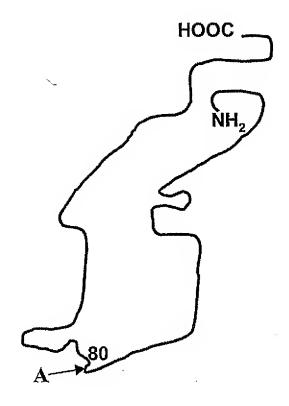
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$$(Fuc)_{i} \\ \mathbf{A} \leftarrow GlcNAc - GlcNAc - Man \\ (R')_{n} \\ (R')_{n} \\ (Fuc)_{i} \\ Man \\ [[GlcNAc - (Gal)_{a}]_{e} - (Sia)_{j} - (R)_{v} \\ [[GlcNAc - (Gal)_{b}]_{f} - (Sia)_{k} - (R)_{w} \\ [[GlcNAc - (Gal)_{c}]_{g} - (Sia)_{l} - (R)_{x} \\ [[GlcNAc - (Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y} \\ [[GlcNac - (Gal)_{d}]_{h} - (Sia)_{m} - (R)_{w} \\ [[GlcNac -$$

a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = polymer

FIG. 29A

```
CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 29B

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```
Insect cell expressed IF-beta a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 2. CMP-SA-PEG, ST3Gal3, buffer, salt

```
b, d, f, h, k, m, n, s, u, w, y = 0;
a, c, e, g, i, r, t (independently selected) = 0 or 1;
j, l, v, x (independently selected) = 0 or 1;
z = 1; R = PEG.
```

Yeast expressed IF-beta a-n = 0; z = 1; r-y (independently selected) = 0 to 1; R (branched or linear) = Man, oligomannose or polysaccharide.

- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal
- 3.. CMP-SA-PEG, ST3Gal3

a-m, r-z= 0; n = 1; R' = -Gal-Sia-PEG.

FIG. 29D

```
CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.
```

1. CMP-SA-PEG, ST3Gal3

```
h = 1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

FIG. 29E

Insect cell expressed IF-beta a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

FIG. 29F

Yeast expressed IF-beta a-n = 0; z = 1; r-y (independently selected) = 0 to 1; R (branched or linear) = Man, oligomannose.

- 1. mannosidases
- 2. GNT's 1,2,4,5, UDP-GlcNAc
- 3. Galactosyltransferase, UDP-Gal
- 4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

NSO expressed IF-beta a-i, r-u (independently selected) = 0 or 1; j-m, n, v-y = 0; z = 1.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H₄N₂-PEG

a-i, j-m, r-y (independently selected) = 0 or 1; n = 0; z = 1; R = PEG.

FIG. 29H

CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.

1. CMP-SA-PEG, α2,8-ST

h = 1 to 3; a-g, i, r-u (independently selected) = 0 or 1; j-m (independently selected) = 0 to 2; v-y (independently selected) = 1, when j-m (independently selected) is 2; z = 1; n = 0; R = PEG.

FIG. 291

CHO, BHK, 293 cells, Vero expressed IF-beta a-g, j-m, r-u (independently selected) = 0 or 1; h = 1 to 3; n, v-y = 0; z = 1.

- 1. Sialidase
- 2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1; h = 1 to 3; n = 0; z = 1; R = PEG.

FIG. 29J

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n=0; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 29K

```
NSO expressed Ifn-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0;
Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. α-Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; R = PEG
n = 0; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
```

FIG. 29L

```
CHO, BHK, 293 cells, Vero expressed Ifin-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h=1; n, v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n = 0;
v-y (independently selected) = 0 or 1; R = PEG.
```

FIG. 29M

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

1. CMP-SA-levulinate, ST3Gal3, buffer, salt
2. H₄N₂-PEG

a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n = 0; v-y (independently selected) = 0 or 1; R = PEG.

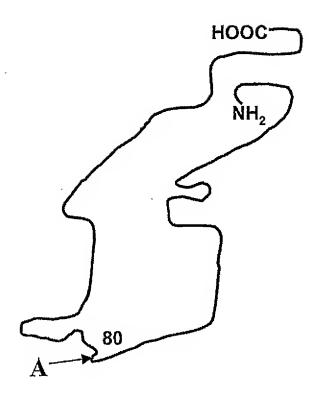
FIG. 29N

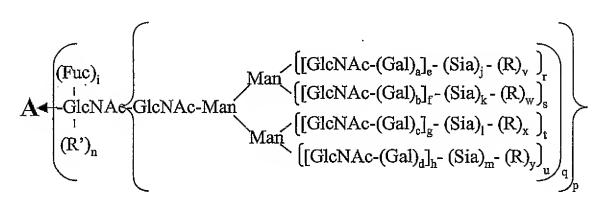
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, r-u, z (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; n, v-y (independently selected) = 0.

FIG. 290





a-d, i, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group; R' = H, glycosyl group, modifying group, glycoconjugate.

```
Insect cell expressed Ifn-beta.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 29Q

```
Yeast expressed Ifn-beta.

a-m=0; q-y (independently selected) = 0 to 1;

p=1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0;
n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.
```

FIG. 29R

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal3, desialylated transferrin.
- 3. CMP-SA, ST3Gal3

```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

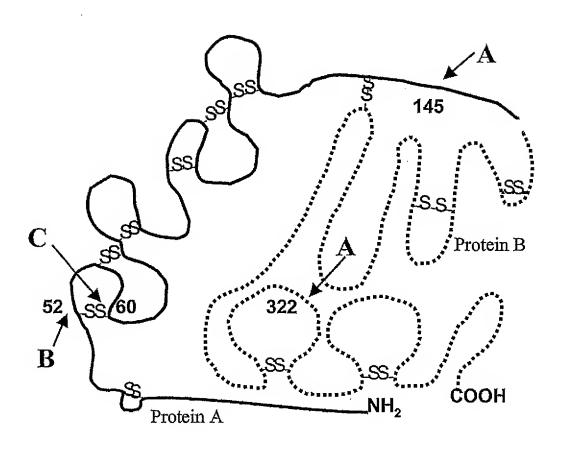
R = linker-transferrin.
```

FIG. 29S

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$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_a]_e - (\mathrm{Sia})_j - (\mathrm{R})_v \end{bmatrix}_r \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_k - (\mathrm{R})_w \end{bmatrix}_s \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_g - (\mathrm{Sia})_l - (\mathrm{R})_x \end{bmatrix}_t \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_l - (\mathrm{R})_x \end{bmatrix}_t \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_h]_h - (\mathrm{Gal})_h - (\mathrm{Gal})_h - (\mathrm{Gal})_h -$$

$$\mathbf{B} \leftarrow \left(\text{Glc-}(Xyl)_n \right)_n$$

$$\mathbf{C}$$
 \leftarrow [-Fuc]_p

a-d, i, q-u (independently selected) = 0 or 1. o, p (independently selected) = 0 or 1. e-h, n (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-y = 0; R = modifying group, mannose, oligomannose, Sia-Lewis X, Sia-Lewis A..

FIG. 30A

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BHK expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mole eq), ST3Gal3

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1;
r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4;
v, x, (independently selected) = 1,
when j, l (respectively, independently selected) is 1;
R = PEG.
```

FIG. 30B

CHO, BHK, 293 cells, Vero expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, v-y=0; n=0-4.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mole eq), ST3Gal3
- 3. CMP-SA (8 mol eq), ST3Gal3

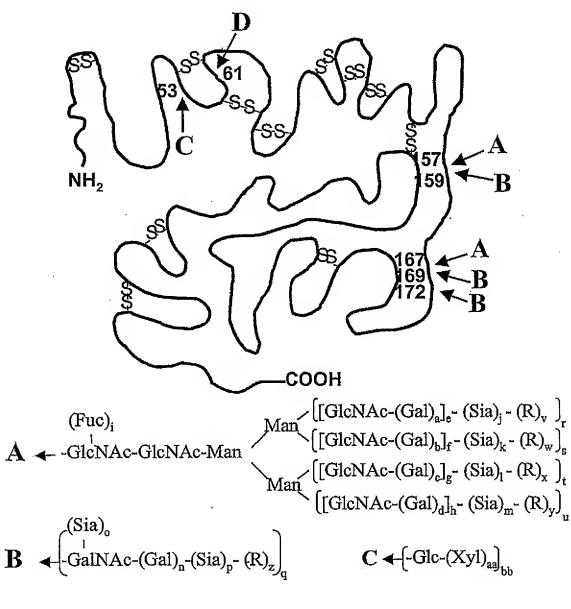
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4; v or x, (independently selected) = 1, when j or l, (respectively, independently selected) is 1; R = PEG.

```
NSO expressed Factor VII or VIIa
a--u (independently selected) = 0 or 1;
v-y = 0; n = 0-4;
Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, o-u (independently selected) = 0 or 1;
n = 0-4; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
Sia = Sia; R = PEG.
```

FIG. 30D



$$\mathbf{D} \leftarrow \text{-Fuc}\left\{-(\text{GlcNAc})_{\text{cc}} - (\text{Gal})_{\text{dd}} - (\text{Sia})_{\text{ee}}\right\}_{\text{ff}} - (R)_{\text{gg}}$$

a-d, i, n-u (independently selected) = 0 or 1.
bb, cc, dd, ee, ff, gg (independently selected) = 0 or 1.
e-h, aa (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 20.
v-z = 0; R = modifying group, mannose, oligo-mannose.

FIG. 31A

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1;
o, p, z = 0;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
R = PEG.
```

FIG. 31B

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3
- 3. ST3Gal1, CMP-SA

```
a-d, n, p, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1;
j-m, ee, v-y, gg (independently selected) = 0 or 1;
o, z = 0; R = PEG.
```

FIG. 31C

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CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

- 1. sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- 4. CMP-SA-PEG, ST3Gal1

```
a-d, n, q = 1; e-h = 1 to 4;

aa, bb, cc, dd, ee, ff, i, r-u (independently selected) =

0 or 1; R = PEG;

o, v-y, gg = 0;

j-m, p, ee (independently selected) = 0 or 1, but when

p = 1, z = 1.
```

FIG. 31D

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

CMP-SA-PEG, ST3Gal3

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1; R = PEG;
o, p, z = 0; j-m, ee, v-y, gg (independently selected) =
0 or 1.
```

FIG. 31E

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H.N. PEC

2. H₄N₂-PEG

```
a-d, q = 1; e-h = 1 to 4;

aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)

= 0 or 1;

o, p, z = 0; R = PEG;

j-m, ee, v-y, gg (independently selected) = 0 or 1.
```

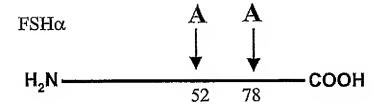
FIG. 31F

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.
```

1. CMP-SA-PEG, α 2,8-ST

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)
= 0 or 1;
o, p, z = 0; R= PEG;
j-m, ee (independently selected) = 0 to 2;
v-y, gg (independently selected) = 1, when j-m (independently selected) is 2;
```

FIG. 31G



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & \operatorname{Man} \left([\operatorname{GlcNAc-(Gal)}_{a}]_{e^{-}} (\operatorname{Sia})_{j^{-}} (\operatorname{R})_{v} \right)_{r} \\ (\operatorname{GlcNAc-GlcNAc-Man} & \left[[\operatorname{GlcNAc-(Gal)}_{b}]_{f^{-}} (\operatorname{Sia})_{k^{-}} (\operatorname{R})_{w} \right]_{s} \\ \operatorname{Man} \left([\operatorname{GlcNAc-(Gal)}_{d}]_{g^{-}} (\operatorname{Sia})_{l^{-}} (\operatorname{R})_{x} \right)_{t} \\ \left([\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \right)_{u} \\ \operatorname{GlcNAc-(Gal)}_{d} \left(\operatorname{Sia})_{m^{-}} (\operatorname{R})_{u} \right)_{u} \\ \operatorname{GlcNAc-(Gal)}_{d} \left(\operatorname{Sia})_{u} \right)_{u} \\ \operatorname{GlcNAc-(Gal)}_{u} \left(\operatorname{Sia})_{u} \right)_{u} \\ \operatorname{GlcNAc-($$

a-d, i, q-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose.

FIG. 32A

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.

FIG. 32B

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG. WO 03/031464 PCT/US02/32263

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NSO expressed FSH.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.

- 1. Sialidase and α-galactosidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal1

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

FIG. 32D

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 32E

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H₄N₂-PEG

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 32F

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 32G

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```
Insect cell expressed FSH.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 32H

```
Yeast expressed FSH.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0;
n (independently selected) = 0 or 1;
R' = -Gal-Sia-PEG.
```

```
CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal1, desialylated chorionic gonadrophin (CG) produced in CHO.
- 3. CMP-SA, ST3Gal3, ST3Gal1

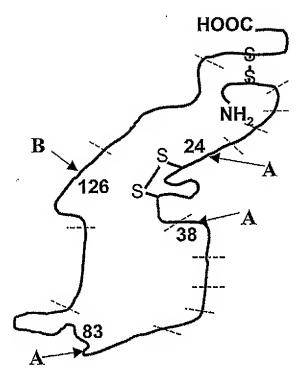
```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-CG.
```

FIG. 32J



$$\mathbf{A} \leftarrow \text{-GlcNAc-GlcNAc-Man} \qquad \begin{bmatrix} [\text{GlcNAc-(Gal)}_a]_e^- & (\text{Sia)}_j^- & (\text{R)}_v \end{bmatrix}_r \\ [\text{GlcNAc-(Gal)}_b]_f^- & (\text{Sia)}_k^- & (\text{R)}_w \end{bmatrix}_s \\ \mathbf{A} \leftarrow \text{-GlcNAc-GlcNAc-Man} \qquad \begin{bmatrix} [\text{GlcNAc-(Gal)}_c]_g^- & (\text{Sia)}_l^- & (\text{R)}_x \end{bmatrix}_t \\ [\text{GlcNAc-(Gal)}_d]_h^- & (\text{Sia)}_m^- & (\text{R)}_y \end{bmatrix}_u \\ \mathbf{B} \leftarrow \begin{bmatrix} (\text{Sia)}_o & (\text{Sia)}_p^- & (\text{R)}_z \end{bmatrix}_t \\ [\text{GalNAc-(Gal)}_n^- & (\text{Sia)}_p^- & (\text{R)}_z \end{bmatrix}_t$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. v-z = 0;

R = modifying group, mannose, oligo-mannose.

FIG. 33A

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; v-z = 0
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

FIG. 33B

```
Insect cell expressed EPO
a-d, f, h, j-q, s, u, v-z = 0;
e, g, i, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- 2. CMP-SA-PEG, ST3Gal3

b, d, f, h, k, m-q, s, u, w, y, z = 0; a, c, e, g, i, r, t (independently selected)= 0 or 1; j, l, v, x (independently selected) = 0 or 1; R = PEG.

CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

- 1. sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- 4. CMP-SA-PEG, ST3Gal1

```
a-h, n, q = 1;
i-m, o, r-u (independently selected) = 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.
```

FIG. 33D

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z = 0
```

1. CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

FIG. 33E

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Insect cell expressed EPO a-d, f, h, j-m, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

a-c, e-g, n, q-t, v-x, z (independently selected) = 0 or 1; d, h, j-m, o, p, y, z = 0; R = PEG.

FIG. 33F

Insect cell expressed EPO a-d, f, h, j-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- 2. Galactosidase (synthetic enzyme), PEG-Gal-F.

a-c, e-g, n, q-t, v-x, z (independently selected) = 0 or 1; d, h, j-m, o, p, y, z = 0; R = PEG.

FIG. 33G

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```
NSO expressed NESP
q = 1; a-i, n, r-u (independently selected) = 0
or 1; j-m, o, p, v-z = 0

1. CMP-SA-levulinate, ST3Gal3,
buffer, salt

2. H<sub>4</sub>N<sub>2</sub>-PEG

q = 1; a-i, j-n, r-y (independently selected) =
0 or 1;
o, p, z = 0; R = PEG.
```

FIG. 33H

```
CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z = 0
```

1. CMP-SA-PEG, α 2,8-ST

```
a-g, n, q = 1; h = 1 to 3;

i, o, p (independently selected) = 0 or 1;

r-u (independently selected) = 0 to 1;

j-m (independently selected) = 0 to 2;

v-y (independently selected) = 1,

when j-m (independently selected) is 2;

R = PEG; z = 0.
```

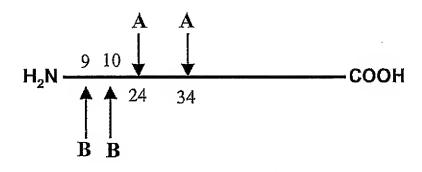
FIG. 331

CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; v-z = 0

1. CMP-SA, poly- α 2,8-ST

a-g, n, q = 1; h = 1 to 3; i, j-m, o, p, r-u, (independently selected) = 0 or 1; v-z (independently selected) = 0-40; R = Sia.

FIG. 33J



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} \\ -\operatorname{GlcNAc-GlcNAc-Man} \end{bmatrix} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{a}]_{e^{-}} (\operatorname{Sia})_{j^{-}} (\operatorname{R})_{v} \\ [\operatorname{GlcNAc-(Gal)}_{b}]_{f^{-}} (\operatorname{Sia})_{k^{-}} (\operatorname{R})_{w} \end{bmatrix}_{s} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{b}]_{g^{-}} (\operatorname{Sia})_{l^{-}} (\operatorname{R})_{x} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u} \\ \operatorname{Man} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{m^{-}} (\operatorname{R})_{m^{-}} (\operatorname{R})_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{m^{-}} (\operatorname{R})_{$$

a-d, i, n-u, aa (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 34A

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CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 34B

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, as (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3 & ST3Gal1

a-d, i-m, p-u, aa (independently selected) = 0 or 1; o, z = 0; n, e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 34C

NSO expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0; Sia (independently selected) = Sia or Gal.

- 1. Sialidase and α-galactosidase
- 2. CMP-SA, ST3Gal3
- 2. CMP-SA-PEG, ST3Gal1

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1; n, e-h = 1; o, v-y = 0; z = 1, when p = 1; R = PEG.

FIG. 34D

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, q-y, as (independently selected) = 0 or 1; o, p, z = 0; n, e-h = 1; R = PEG.

FIG. 34E

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, as (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- CMP-SA-levulinate, ST3Gal3, buffer, salt
 HAL BEG
- 2. H₄N₂-PEG

a-d, i-m, o-y, aa (independently selected) = 0 or 1; z = 0; n, e-h = 1; R = PEG.

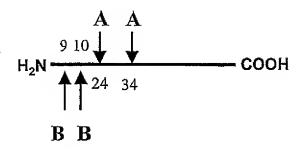
FIG. 34F

CHO, BHK, 293 cells, Vero expressed GMCSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.

1. CMP-SA, α2,8-ST

a-d, i, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; j-m (independently selected) = 0-20; v-z (independently selected) = 0.

FIG. 34G



$$\mathbf{A} \leftarrow \begin{array}{c} \text{(Fuc)}_{i} \\ \text{-GlcNAc-Man} \\ \text{(R')}_{cc} \end{array} \qquad \begin{array}{c} \text{(IGlcNAc-(Gal)}_{a}]_{e^{-}} \text{(Sia)}_{j^{-}} \text{(R)}_{v} \\ \text{[IGlcNAc-(Gal)}_{b}]_{f^{-}} \text{(Sia)}_{k^{-}} \text{(R)}_{w} \\ \text{[IGlcNAc-(Gal)}_{c}]_{g^{-}} \text{(Sia)}_{l^{-}} \text{(R)}_{x} \\ \text{[IGlcNAc-(Gal)}_{d}]_{h^{-}} \text{(Sia)}_{m^{-}} \text{(R)}_{y} \\ \text{bb} \end{array}$$

$$\mathbf{B}$$

$$\begin{array}{c}
\text{(Sia)}_{0} \\
\text{-GalNAc-(Gal)}_{n}\text{-(Sia)}_{p}\text{- (R)}_{z}
\end{array}$$

a-d, i, n-u, aa, bb, cc (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose. R'= H, glycosyl residue, modifying group. glycoconjugate.

FIG. 34H

```
Insect cell expressed GM-CSF. a-d, f, h, j-m, o, p, s, u, v-z=0; e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, n, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 341

```
Yeast expressed GM-CSF.

a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1;
bb = 1; R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

- 1. Endoglycanase
- 2. mannosidase (if aa = 1).
- 3. Galactosyltransferase, UDP-Gal-PEG

```
a-p, r-z, aa, bb = 0;
q, cc (independently selected) = 0 or 1;
R' = -Gal-PEG.
```

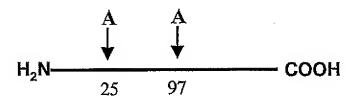
FIG. 34J

CHO, BHK, 293 cells, Vero expressed GM-CSF. a--m, o-u, aa, bb (independently selected) = 0 or 1; n, v-z, cc = 0.

- 1. sialidase
- 2. CMP-SA, ST3Gal3
- 2. CMP-SA-linker-SA-CMP, ST3Gal1
- 3. ST3Gal3, transferrin

a--m, p-u, z, as (independently selected) = 0 or 1; o, v-y, cc = 0; bb, n = 1; R = transferrin.

FIG. 34K



$$\mathbf{A} \leftarrow \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e^{-}} \left(\operatorname{Sia} \right)_{j} - \left(\operatorname{R} \right)_{v} \right)_{r} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f^{-}} \left(\operatorname{Sia} \right)_{k} - \left(\operatorname{R} \right)_{w} \right]_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{c} \right]_{g^{-}} \left(\operatorname{Sia} \right)_{l} - \left(\operatorname{R} \right)_{x} \right)_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right)_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right)_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ \left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} \left(\operatorname{R} \right)_{y} \right]_{u} \\ \left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{u} \\ \left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{u} \left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{u} \\ \left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{u}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 35A

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 35B

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

```
NSO expressed Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. α-Galactosyltransferase, UDP-Gal
- **▼** 3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 35D

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 35E

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. CMP-SA-levulinate, ST3Gal3,
- 2. H₄N₂-PEG

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

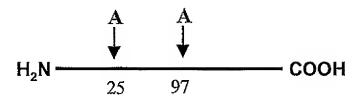
FIG. 35F

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

1. CMP-SA, α 2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 35G



$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_a]_e - (\mathrm{Sia})_j - (\mathrm{R})_v \end{bmatrix}_r \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_k - (\mathrm{R})_w \end{bmatrix}_s \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_g - (\mathrm{Sia})_l - (\mathrm{R})_x \end{bmatrix}_t \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_l - (\mathrm{R})_x \end{bmatrix}_t \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_l - (\mathrm{R})_x \end{bmatrix}_t \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Gal})_d \end{bmatrix}_u = \mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_h]_h - (\mathrm{Gal})_h - (\mathrm{Gal})_h - (\mathrm{Gal})_h - (\mathrm{Gal})$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 35H

```
Insect or fungi cell expressed IF-gamma.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 351

```
Yeast expressed IF-gamma.

a-m = 0; q-y (independently selected) = 0 to 1; p = 1;

R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

FIG. 35J

CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase.

```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 35K

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1; n, v-y = 0.
```

1. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1;
n, v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 35L

```
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

1. GNT's 1 & 2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, q (independently selected) = 0 or 1;
p = 1; v, x (independently selected) = 1,
when e, g (independently selected) is 1;
R = PEG.
```

Insect or fungi cell expressed IF-gamma.

FIG. 35M

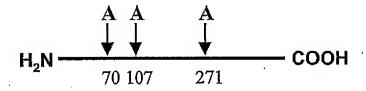
```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-PEG, α2,8-ST
```

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-2;
v-y (independently selected) = 1,
when j-m (independently selected) = 2;
R = PEG.
```

FIG. 35N

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$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_a]_{e^-} & (\mathrm{Sia})_j - (\mathrm{R})_v \end{bmatrix}_r \\ - (\mathrm{GlcNAc} - \mathrm{GlcNAc} - \mathrm{Man} \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_k - (\mathrm{R})_w \end{bmatrix}_s \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_c]_g - (\mathrm{Sia})_l - (\mathrm{R})_x \end{bmatrix}_t \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u \\ - (\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \\ - (\mathrm{Gal})_d - (\mathrm{Gal})_$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

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```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub> antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 36B

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub> antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 36C

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```
NSO expressed \alpha_1-antitrypsin.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. α-Galactosyltransferase, UDP-Gal
- ↓ 3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 36D

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed alpha-1 antitrypsin.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 36E

CHO, BHK, 293 cells, Vero or transgenic animal expressed α₁-antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H_4N_2 -PEG

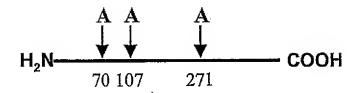
a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 36F

CHO, BHK, 293 cells, Vero expressed α_1 -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.



$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_{a}]_{c} - (\mathrm{Sia})_{j} - (\mathrm{R})_{v} \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_{b}]_{f} - (\mathrm{Sia})_{k} - (\mathrm{R})_{w} \end{bmatrix}_{s} \\ \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_{b}]_{f} - (\mathrm{Sia})_{k} - (\mathrm{R})_{w} \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_{c}]_{g} - (\mathrm{Sia})_{l} - (\mathrm{R})_{x} \end{bmatrix}_{t} \\ \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_{d}]_{h} - (\mathrm{Sia})_{m} - (\mathrm{R})_{y} \end{bmatrix}_{u} \\ q \\ p \\ \end{bmatrix}_{p}$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 36H

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```
Insect or fungi cell expressed \alpha_1-antitrypsin.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1; j-m = 0;
v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 361

```
Yeast expressed \alpha_1-antitrypsin.

a-m=0; q-y (independently selected) = 0 to 1;

p=1; R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

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CHO, BHK, 293 cells, Vero expressed α_1 -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase

```
a-m, q-u (independently selected) = 0 or 1;

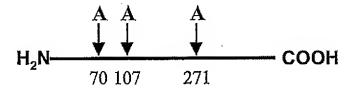
p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 36K

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$$(Fuc)_{i}$$

$$A \leftarrow GlcNAc-Man$$

$$(R')_{p}$$

$$(R')_{q}$$

$$(Fuc)_{i}$$

$$[[GlcNAc-(Gal)_{a}]_{e}-(Sia)_{j}-(R)_{v}$$

$$[[GlcNAc-(Gal)_{b}]_{f}-(Sia)_{k}-(R)_{w}]_{s}$$

$$[[GlcNAc-(Gal)_{c}]_{g}-(Sia)_{l}-(R)_{x}$$

$$[[GlcNAc-(Gal)_{d}]_{h}-(Sia)_{m}-(R)_{y}]_{u}$$

$$[[GlcNAc-(Gal)_{d}]_{h}-(Sia)_{m}-(R)_{y}]_{u}$$

a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

R = polymer;

R', R" (independently selected) = sugar, glycoconjugate.

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Yeast expressed alpha-1 antitrypsin.
a-h, i-m, p, q = 0;
R (independently selected) = mannose, oligomannose, polymannose;
r-u, v-y (independently selected) = 0 or 1; n, o = 1.

- 1. endoglycanase
- 2. Galactosyltransferase, UDP-Gal-PEG

a-h, i-o, q, r-u, v-y = 0; p = 1. R" = Gal-PEG.

FIG. 36M

Plant expressed alpha-1 antitrypsin. a-d, f, h, j- m, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1; n=1; R' = xylose

- 1. hexosaminidase,
- 2. alpha mannosidase and xylosidase
- 3. GlcNAc transferase, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0; q = 1; R' = GlcNAc-PEG.

FIG. 36N

CHO, BHK, 293 cells, Vero, transgenic animal expressed α₁ antitrypsin.
a-h, i-o, r-u (independently selected) = 0 or 1;
p, q, v-y = 0.

1. CMP-SA-PEG, ST3Gal3

a-h, i-o, r-u (independently selected) = 0 or 1; p, q = 0; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 360

$$A = \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e} - \left(\operatorname{Sia} \right)_{j} - \left(\operatorname{R} \right)_{v} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f} - \left(\operatorname{Sia} \right)_{k} - \left(\operatorname{R} \right)_{w} \right]_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{c} \right]_{g} - \left(\operatorname{Sia} \right)_{l} - \left(\operatorname{R} \right)_{x} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h} - \left(\operatorname{Sia} \right)_{m} - \left(\operatorname{R} \right)_{y} \right]_{u} \end{bmatrix}_{q}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 37A

CHO, BHK, 293 cells, Vero expressed Cerezyme a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 37B

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-M-6-P (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = mannose-6-phosphate

```
NSO expressed Cerezyme.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. α-Galactosyltransferase, UDP-Gal
- **★** 3. CMP-SA-M-6-P, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = mannose-6 phosphate
```

FIG. 37D

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = Mannose-6-phosphate
```

FIG. 37E

CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H₄N₂-spacer-M-6-P or clustered M-6-P

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = M-6-P or clustered M-6-P
```

FIG. 37F

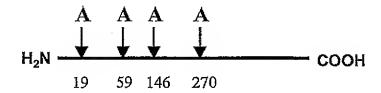
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

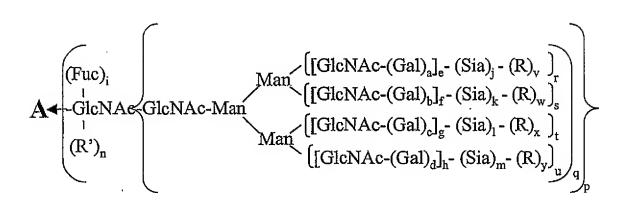
1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 37G

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a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 37H

```
Insect cell expressed Cerezyme.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0;
v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 371

```
Yeast expressed Cerezyme.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, p-y=0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

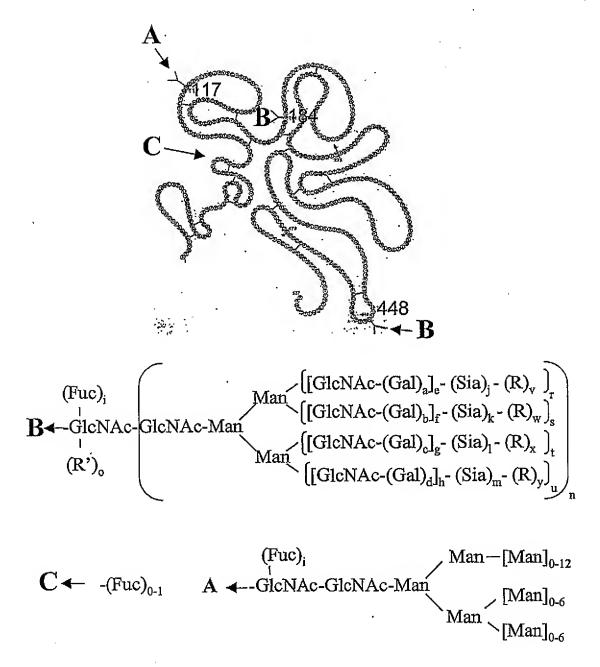
FIG. 37J

CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
 - 2. ST3Gal3, desialylated transferrin.
 - 3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-transferrin.

FIG. 37K



a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 38A

147/345

```
CHO, BHK, 293 cells, Vero expressed tPA a-g, n = 1; h = 1 to 3; j-m, i, (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; o, v-y = 0.
```

- 1. Mannosidase(s), sialidase
- 2. GNT1,2 (4 and/or 5) UDP-GlcNAc
- 3. Gal transferase, UDP-Gal
- 4. CMP-SA-PEG, ST3Gal3

```
A = B; a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1;
o = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG
```

FIG. 38B

```
Insect or fungi cell expressed tPA
A = B; a-d, f, h, j-o, s, u, v-y = 0;
e, g, i, n, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- ♦ 3. CMP-SA-PEG, ST3Gal3

```
A = B; b, d, f, h, k, m, o, s, u, w, y = 0;
a, c, e, g, i, r, t (independently selected) = 0 or 1;
n = 1; j, l, v, x (independently selected) = 0 or 1;
R = PEG.
```

FIG. 38C

Yeast expressed tPA B = A; i = 0.

- 1. endoglycanase
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

FIG. 38D

Insect or fungi cell expressed tPA A = B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. alpha and beta mannosidases
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

Insect or fungi cell expressed tPA A = B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

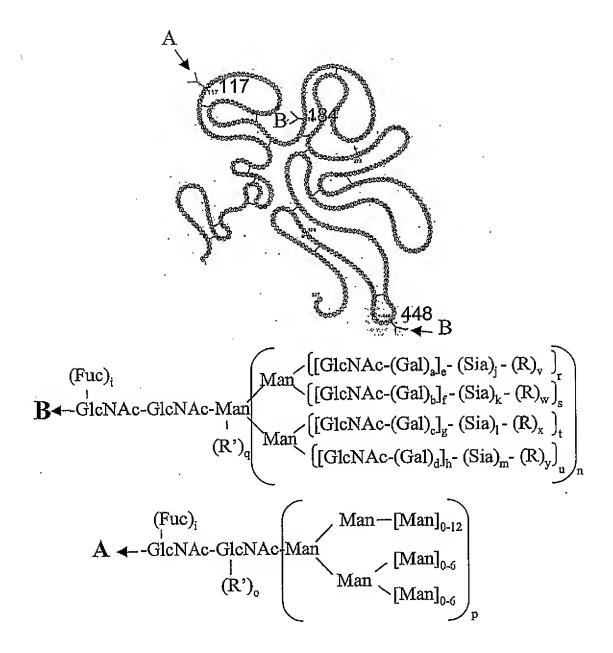
A = B; b, d, f, h, j-o, s, u, w, y = 0; a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1; n = 1; R = PEG.

FIG. 38F

Insect or fungi cell expressed tPA A = B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. GNT's 1 & 2, UDP-GlcNAc
- 2. Galactosidase (synthetic enzyme), PEG-Gal-F.

A = B; b, d, f, h, j-o, s, u, w, y = 0; a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1; n = 1; R = PEG.



a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 38H

```
NSO expressed tPA

A = B; a-m, r-u (independently selected) = 0 or 1;

n = 1; o, p, q, v-y = 0
```

- 1. sialidase, alpha-galactosidase
- 2. CMP-SA-levulinate, ST3Gal3,
- $3. H_4 N_2$ -PEG

```
A = B; a-m, r-y (independently selected) = 0 or 1;

n = 1; o, p, q = 0;

v-y (independently selected) = 1,

when j-m (independently selected) is 1;

R = PEG.
```

FIG. 381

```
CHO, BHK, 293 cells, Vero expressed tPA a-g, n, p = 1; h = 1 to 3; j-m, i, (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; q, o, v-y = 0.
```

- 1. alpha and beta Mannosidases
- 2. CMP-SA, ST3Gal3
- 3. Galactosyltransferase, UDP-Gal-PEG

```
a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1; o = 1;
q, p, v-y = 0; j-m (independently selected) = 0 or 1;
R' = Gal-PEG
```

FIG. 38J

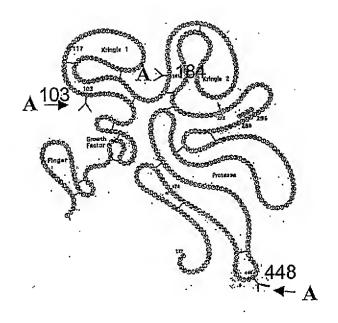
```
Plant expressed tPA

A = B; a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1;
n = 1; R' = xylose
```

- 1. hexosaminidase,
- 2. alpha mannosidase and xylosidase
- 3. GlcNAc transferase, UDP-GlcNAc-PEG

```
A = B; a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0;
q = 1; R' = GlcNAc-PEG.
```

FIG. 38K



$$\mathbf{A} \leftarrow \begin{bmatrix} \left[\operatorname{GlcNAc-(Gal)}_{a} \right]_{e^{-}} \left(\operatorname{Sia} \right)_{j^{-}} - \left(\operatorname{R} \right)_{v^{-}} \right]_{r} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{f^{-}} \left(\operatorname{Sia} \right)_{k^{-}} - \left(\operatorname{R} \right)_{w^{-}} \right]_{s} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{b} \right]_{g^{-}} \left(\operatorname{Sia} \right)_{l^{-}} - \left(\operatorname{R} \right)_{w^{-}} \right]_{t} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right]_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{y^{-}} \right)_{u^{-}} \\ \left[\left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{m^{-}} - \left(\operatorname{R} \right)_{u^{-}} \right)_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left(\operatorname{Sia} \right)_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \right]_{u^{-}} \\ \left[\operatorname{GlcNAc-(Gal)}_{d^{-}} \right]_{u^{-}}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 38L

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

FIG. 38M

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 38N

```
NSO expressed TNK tPA

a-d, i-m, q-u (independently selected) = 0 or 1;

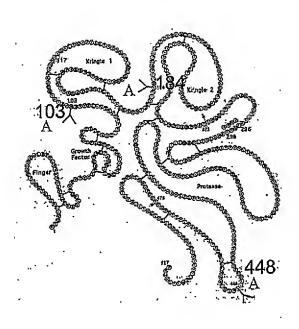
e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 380



$$\mathbf{A} \leftarrow \begin{bmatrix} [\mathrm{GlcNAc} - (\mathrm{Gal})_a]_c - (\mathrm{Sia})_j - (\mathrm{R})_v \end{bmatrix}_r \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_b]_f - (\mathrm{Sia})_k - (\mathrm{R})_w \end{bmatrix}_s \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_c]_g - (\mathrm{Sia})_l - (\mathrm{R})_x \end{bmatrix}_t \\ [\mathrm{GlcNAc} - (\mathrm{Gal})_d]_h - (\mathrm{Sia})_m - (\mathrm{R})_y \end{bmatrix}_u$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

FIG. 38Q

```
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H₄N₂-PEG

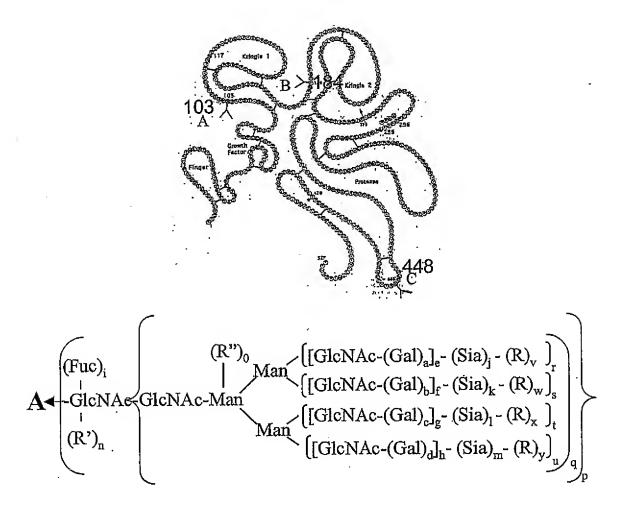
a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 38S



a-d, i, n-y (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group, glycoconjugate.
R" = glycosyl residue.

```
Insect cell expressed TNK tPA
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

FIG. 38U

```
Yeast expressed TNK tPA
a-m = 0; q-y (independently selected) = 0 to 1; p = 1;
R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-m, p-y=0; n (independently selected) = 0 or 1; R' = -Gal-PEG.
```

FIG. 38V

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, anti-TNF IG chimera produced in CHO.

a-m, r-u (independently selected) = 0 or 1; p, q = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-anti-TNF IG chimera protein.

FIG. 38W